Quantitative Proteomics: Ovalbumin Detection Using Forward-Phase Protein Microarrays and Suspension Arrays

NIST is conducting studies to enable better proteomics measurements with protein microarrays, an important emerging technology. Comparison of results obtained from the 2 major formats of high-throughput protein microarrays against conventional gel electrophoresis, will allow for the understanding of such data and enable more rapid adaptation of this high-throughput technology into the marketplace.

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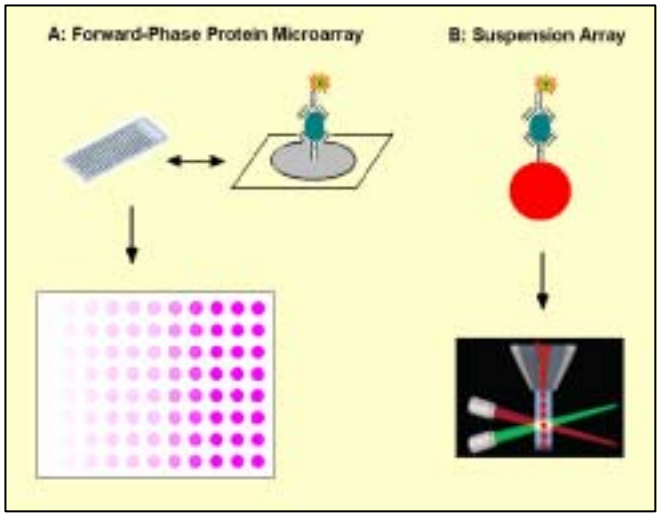
Protein microarray technology is an emerging proteomic tool with broad and unique applications for quantitative analysis and discovery. Compared to other proteomic technologies, such as mass spectrometry (MS), 2-dimensional polyacrilamide gel electrophoresis (2D-PAGE), suspension arrays, micro-ELISA, and multiplexed immunoassays, protein microarrays are very high-throughput and uniquely capable of investigating intermolecular interactions between protein-protein, protein-DNA, protein-RNA, and protein-cell. Protein microarrays are therefore becoming extremely useful tools for profiling cellular signal pathways and networks of clinical samples.

At present, there are two major formats of forward phase protein microarrays, printed arrays and suspension (non-geometric) arrays. Printed arrays contain affinity ligands (usually antibodies) spotted on a solid matrix and suspension arrays are comprised of microbeads coated with tha affinity ligands. In the forward phase array, high affinity antibodies are arrayed and a test samples is added to the array. Analysis of the array shows the types of analytes present in the test sample. Test samples are incubated with one type of antibody. By screening a large number of sample arrays against a number of antibodies, the final readings are directly compared across results from multiple samples.

NIST is advancing overall proteomics efforts by developing knowledge of the advantages and disadvantages of protein microarrays, as compared to 2D-PAGE proteomic analysis. These efforts are expected to allow the more rational clinical use of these important proteomics tools.

In the present study, ovalbumin is used as a model system to simulate ricin and botolinum toxins. Reliability, comparability, dynamic range, and linearity of printed arrays were examined against suspension arrays. Both formats utilized a high affinity, commercially available polyclonal antibody as the affinity ligand. Ovalbumin samples with two different purities, 38.0 % and 76.0 % (w/w), as determined by polyacrylamide gel electrophoresis (PAGE), were used as the analyte. These samples were used to evaluate the effect of sample purity on detection.



It was found that the solid-phase, printed protein microarrays had a dynamic range of four orders of magnitude and a sensitivity of less than 1 pg/mL. The dynamic range and sensitivity of suspension arrays were close to two orders of magnitude and 0.25 ng/mL. The sensitivity we observed for the suspension arrays is comparable to that reported for enzyme-linked immunosorbent assays (ELISA) in the literature. The data obtained from the solid-phase printed protein arrays gave values that were consistent with the PAGE data. The data from the suspension arrays were not as consistent and may indicate that this format may not give as reliable data with impure samples.

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